RESEARCH ARTICLE

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The Relationship between *SALL4* and *Fascin* Expression and the Progression of Colorectal Carcinoma

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Abstract

Objective: *SALL4* (Spalt-like transcription factor 4) is a stem cell transcription factor that is reactivated in various tumor tissues and has a proven pro-metastatic role in colorectal cancer (CRC). However, the mechanism of its reactivation in CRC remains elusive. *fascin* is an actin-bundling protein linked to CRC progression. Independent of this activity, *fascin* regulates stemness and embryonic stem cell-related genes in some cancers. Data on the role of *fascin* in regulating stem cell transcription factors in CRC are scarce, and the relationship between *fascin* and *SALL4* expression in CRC has not been investigated. This study aims to evaluate the immunohistochemical expression of *SALL4* and *fascin* in fifty-four CRC cases and their relationship with clinicopathological parameters. **Methods:** The expression of *SALL4* and Fascin determined using immunohistochemistry in 54 paraffin-embedded colectomy specimens from patients with primary colorectal adenocarcinoma. **Result:** *SALL4*-positive expression was detected in 9 cases (16.7%), while moderate-to-high *fascin* expression was found in 21 cases (38.9%). A significant positive relationship was identified between *SALL4* expression and lymphovascular invasion (LVI) (P = 0.033). Moderate-to-high *fascin* expression was significantly associated with advanced pathological tumor (pT) stage (P = 0.023), lymph node (LN) spread (P = 0.031), and LVI (P = 0.010). Furthermore, a significant association was observed between *SALL4* positivity and moderate-to-high *fascin* expression (P = 0.009). **Conclusion:** This is the first study to demonstrate a significant association between *SALL4* and *fascin* expression in CRC patients, suggesting a potential interplay between them. Both proteins may represent potential markers of CRC progression. Molecular studies are required to further investigate the interaction between *SALL4* and *fascin* in CRC.

Keywords: *SALL4- Fascin-* Colorectal cancer

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Introduction

Colorectal cancer (CRC) is the third most frequent cancer and the second leading cause of malignancy-related death worldwide [1]. Strong evidence suggests that neoplasms originate and progress with the aid of cancer stem cells (CSCs), which exhibit features of normal stem cells, such as self-renewal and resistance to therapy. Investigating the mechanisms that regulate and maintain CSCs is, therefore, essential [2].

SALL4, a transcription factor related to the SALL gene family, serves as a CSC marker in various tumors [3, 4]. It is highly expressed in the serum and tissues of CRC, and its expression level correlates with lymph node spread [5]. However, the underlying mechanism of *SALL4* expression in CRC remains unclear [3].

fascin is a 55 kDa actin-binding protein that stabilizes cell protrusions like filopodia. Its expression in neoplastic cells increases migration, infiltration, and lymph node spread [6]. Several actin-independent, pro-metastatic roles of *fascin* have also been reported. For example, *fascin* maintains or increases cancer stemness in melanoma and mammary CSCs independently of its actin-binding function [2, 7, 8].

Data on the role of *fascin* in regulating transcription factors associated with pluripotency and self-renewal in CRC are limited. Moreover, the relationship between *SALL4* and *fascin* in CRC has not been described. Therefore, this study aims to investigate the relationship between *SALL4* and *fascin* expression in CRC and their association with clinicopathological features using immunohistochemistry.

Materials and Methods

Study design and setting

A cross-sectional retrospective study was conducted in the pathology laboratory of Al-Zahraa University Hospital from September 2023 to September 2024.

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Study materials

The study included fifty-four colectomy specimens of primary colorectal adenocarcinoma, comprising 36 cases from female patients and 18 from male patients.

Inclusion and Exclusion criteria

Colectomy specimens of primary colorectal carcinoma from treatment-naïve patients were included. Endoscopic biopsies, cases with inadequate clinical information, and cases that had undergone neoadjuvant therapy were excluded.

Methods

Cases

We included 54 paraffin-embedded colectomy specimens from patients with primary colorectal adenocarcinoma. Multiple sequential 4-µm-thick sections were cut; one was stained with hematoxylin and eosin for re-evaluation, and others were mounted on charged slides for immunohistochemical staining of *fascin* and *SALL4*. Patient data, including age, gender, histopathologic type, grade, LVI, perineural invasion (PNI), pT stage, and nodal status, were collected and analyzed according to the AJCC TNM staging system (8th edition).

Immunohistochemistry

Immunostaining was performed using the standard biotin-avidin technique with anti-*fascin* 1 (55k-2, mouse monoclonal, Ventana) and anti-*SALL4* (6E3, mouse monoclonal, Ventana) antibodies.

Evaluation of immunohistochemistry

SALL4 expression was identified as brownish nuclear staining in tumor cells (Figure 1). Staining intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive tumor cells was graded as: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%). The final score was the sum of the intensity and percentage scores, with a score ≥ 4 considered positive. Testicular seminoma served as a positive control [3].

fascin expression was identified as brownish, granular cytoplasmic or membranous staining (Figure 2). Intensity was graded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The percentage of positive carcinoma cells was graded as: 0 (0%), 1 (<10%), 2 (10-50%), 3 (51-80%), and 4 (>80%). The final immunoreactivity score (IRS) was the product of the intensity and percentage scores, classified as negative (0), low (1-4), intermediate (6-8), and high (9-12). Blood vessel endothelium in the tumor stroma served as an internal positive control [9]. For analysis, cases were grouped as negative/low or moderate/high expression.

For both markers, the primary antibody was omitted for negative controls. Two authors independently and blindly assessed all immunostaining.

Statistical analysis

Data were analyzed using SPSS version 28 (IBM, USA). All data were categorical except for age. Data are presented as numbers and percentages and were compared using the chi-square or Fisher's exact test. All tests were two-sided, with $P < 0.05$ considered significant. The Oxford Handbook of Medical Statistics (2nd ed., 2020) was consulted [10].

Results

The study included 54 cases. Most patients (77.8%) were over 50 years, with a mean age of 56.81 (± 10.65) years. The cohort included 36 females and 18 males.

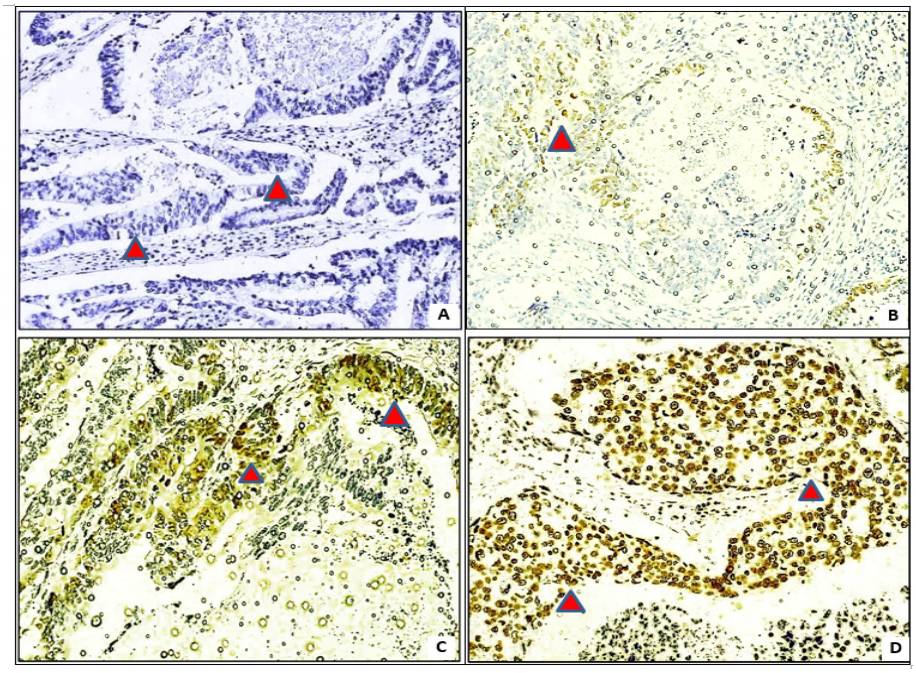

Figure 1. *SALL4* Expression in Colorectal Adenocarcinoma (×200). (A) Negative nuclear staining in GI adenocarcinoma (arrow heads). (B) Weak nuclear staining in GII adenocarcinoma (arrow head). (C) Moderate intensity of nuclear staining in GII adenocarcinoma (arrow heads). (D) Strong intensity of nuclear staining in GIII adenocarcinoma (arrow heads).

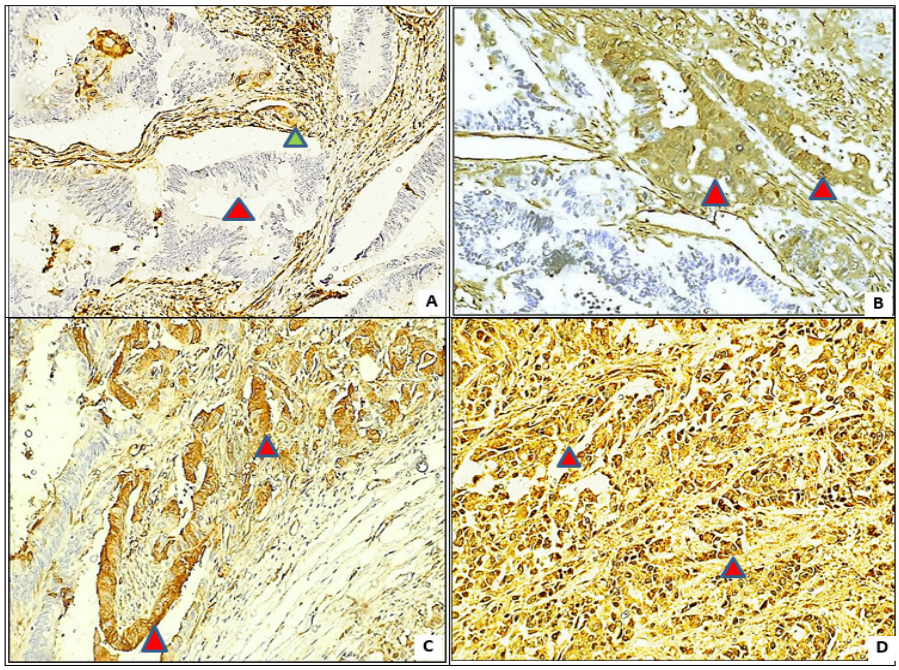

Figure 2. *Fascin* Expression in Colorectal Adenocarcinoma (×200). (A) Negative staining of tumor cells in GI adenocarcinoma (red arrow head) with positive cytoplasmic staining of endothelium of blood vessels of tumor stroma (internal control) (green arrow head). (B) Weak cytoplasmic staining of tumor cells in GII adenocarcinoma (arrow heads). (C) Moderate to strong cytoplasmic & nuclear staining of tumor cells in GII adenocarcinoma (arrow heads). (D) Strong cytoplasmic & nuclear staining of tumor cells in GIII adenocarcinoma (arrow heads).

Table 1. Clinicopathological Data of Cases Included in This Research (no=54).

Parameters	No.	%
Age (years)		
<50 years	12	22.2%
>50 years	42	77.8%
Gender		
Female	36	66.7%
Male	18	33.3%
Tumor type		
Conventional adenocarcinoma	50	92.6%
Mucinous adenocarcinoma	3	5.6%
Signet ring	1	1.9%
Tumor size		
<5cm	28	51.9%
>5cm	26	48.1%
Grade		
Well differentiation	2	3.7%
Moderately differentiated	39	72.2%
Poorly differentiated	13	24.1%
Site		
Right colon	16	29.6%
Transverse colon	2	3.7%
Left colon	32	59.3%
Rectum	4	7.4%
pT-Stage		
T1	1	1.9%
T2	10	18.5%
T3	32	59.3%
T4	11	20.4%
Lymph node metastasis		
N0	20	37.0%
N1	21	38.9%
N2	13	24.1%
LVI		
Negative	16	29.6%
Positive	38	70.4%
PNI		
Negative	41	75.9%
Positive	13	24.1%

Data are expressed in number and (%) No: number; %, percentage; pT, pathological tumor; LVI, lymphovascular invasion; PNI, perineural invasion.

Clinicopathological characteristics are summarized in Table 1.

SALL4 expression was positive in 9 cases (16.7%) and negative in 45 cases (83.3%). *fascin* expression was negative/low in 33 cases (61.1%) and moderate-to-high in 21 cases (38.9%) (Table 2).

A borderline significant relationship was found between *SALL4* expression and tumor grade (P = 0.053), with poorly differentiated tumors more frequent among *SALL4*-positive cases (55.6%) than *SALL4*-negative cases (17.8%). *SALL4* expression was significantly associated with LVI (P = 0.033); all *SALL4*-positive cases (100%) showed LVI, compared to 64.4% of negative cases.

Table 2. *SALL4* and *Fascin* Expression in the Examined Cases

	No.	%
SALL4		
Negative	45	83.3%
Positive	9	16.7%
Fascin		
Negative/Low	33	61.1%
Moderate/High	21	38.9%

Data are expressed in number and (%) No, number; %, percentage

The relationship between *SALL4* and nodal metastasis approached significance (P = 0.06), with 55.6% of positive cases classified as N2 versus 17.8% of negative cases. No significant relationship was found with PNI (P = 0.477) (Table 3).

Table 3. Relation between *SALL4* Expression and Clinicopathological Parameters

	SALL4	
	Positive (No= 9)	Negative (No = 45)
	No. (%)	No. (%)
Grade		
Well-differentiated	0 (0)	2 (4.4)
Moderately differentiated	4 (44.4)	35 (77.8)
Poorly differentiated	5 (55.6)	8 (17.8)
P value	FE 0.053	
Chi-square (χ^2)	5.173	
pT Stage		
pT1 and pT2	1 (11.1)	10 (22.2)
pT3 and pT4	8 (88.9)	35 (77.8)
P value	FE 0.450	
Chi-square (χ^2)	0.571	
L.N metastasis		
N0	2 (22.2)	18 (40)
N1	2 (22.2)	19 (42.2)
N2	5 (55.6)	8 (17.8)
P value	FE0.060	
Chi-square (χ^2)	4.936	
LVI		
Positive	9 (100)	29 (64.4)
Negative	0 (0)	16 (35.6)
P value	FE0.033*	
Chi-square (χ^2)	4.547	
PNI		
Positive	3 (33.3)	10 (22.2)
Negative	6 (66.7)	35 (77.8)
P value	FE0.477	
Chi-square (χ^2)	0.507	

No, number; %, percentage; pT, pathological tumor; L.N, lymph node; LVI, lymphovascular invasion; PNI, perineural invasion. *Significant P-value. FE, Fisher's Exact test

Table 4. Relation between *Fascin-1* Expression and Clinicopathological Parameters

	Fascin	
	Negative/low (No = 33)	Moderate/high (No = 21)
	No. (%)	No. (%)
Grade		
Well-differentiated	2 (6.1)	0 (0)
Moderately differentiated	25 (75.8)	14 (66.7)
Poorly differentiated	6 (18.2)	7 (33.3)
P value	FE0.317	
Chi-square (χ^2)	2.267	
pT Stage		
pT1 and pT 2	10 (30.3)	1 (4.8)
pT3 and pT4	23 (69.7)	20 (94.2)
P value	FE0.023*	
Chi-square (χ^2)	5.161	
L.N metastasis		
N0	15 (45.5)	5 (23.8)
N1	14 (42.4)	7 (33.3)
N2	4 (12.1)	9 (42.9)
P value	0.031*	
Chi-square (χ^2)	6.932	
LVI		
Positive	19 (57.6)	19 (90.5)
Negative	14 (42.4)	2 (9.5)
P value	0.010*	
Chi-square (χ^2)	6.662	
PNI		
Positive	6 (18.2)	7 (33.3)
Negative	27 (81.8)	14 (66.7)
P value	0.204	
Chi-square (χ^2)	1.612	

No, number; %, percentage; pT, pathological tumor; L.N, lymph node; LVI, lymphovascular invasion; PNI, perineural invasion. *Significant P-value. FE, Fisher's Exact test

Moderate-to-high *fascin* expression was significantly associated with advanced pT stage (P = 0.023). The pT3 and pT4 stages were more frequent in the moderate-to-high expression group (94.2%) than in the low-expression group (69.7%). No significant relationship was found

Table 5. Association between *SALL4* and Fascin in the Studied Cases

	SALL4	
Fascin	Negative (No=45)	Positive (No=9)
	No. (%)	No. (%)
Negative/low	31 (68.9)	2 (22.2)
Moderate/high	14 (31.1)	7 (77.8)
P value	FE0.009*	
Chi-square (χ^2)	4.998	

No, number; %, percentage; *Significant P-value. FE, Fisher's Exact test

with tumor grade (P = 0.317). *fascin* expression was significantly associated with lymph node metastasis (P = 0.031) and LVI (P = 0.01). In the low-expression group, 45.5% had no lymph node metastasis (N0), compared to 23.8% in the high-expression group. Conversely, 42.9% of the high-expression group were N2, versus 12.1% of the low-expression group. Regarding LVI, 90.5% of the high-expression group were positive, compared to 57.6% of the low-expression group. No significant relationship was found with PNI (P = 0.204) (Table 4).

A significant association was observed between *SALL4* positivity and moderate-to-high *fascin* expression (P = 0.009). While 68.9% of *SALL4*-negative cases showed low *fascin*, 77.8% of *SALL4*-positive cases showed high *fascin* (Table 5).

Discussion

According to GLOBOCAN projections, new CRC cases are expected to increase globally by 63.3% from 2020 to 2040, underscoring the need for better biomarkers [11]. *SALL4*, a transcription factor and marker of CSCs, has garnered attention for its role in cancer progression [4]. *fascin*, an actin-binding protein, promotes cell invasion and lymph node spread by altering the cytoskeleton [12]. Independently, *fascin* can maintain or increase cancer stemness in certain tumors [2, 7, 8].

In CRC, *fascin* upregulation is crucial for neoplastic cell infiltration and migration [13]. However, its role in regulating pluripotency transcription factors in CRC is unexplored. This study investigated the relationship between *fascin* and the CSC marker *SALL4* in colorectal adenocarcinoma.

We found positive *SALL4* expression in 16.7% of cases. This low frequency aligns with Miettinen et al., who reported rare *SALL4* expression in CRC (<5%) [14]. In contrast, Zhang et al. [3] reported a much higher percentage (73.75%), a discrepancy potentially explained by the use of different antibody clones and detection systems.

We found a borderline significant relationship between *SALL4* positivity and high tumor grade (P = 0.053), consistent with Miettinen et al., who noted that *SALL4*-positive carcinomas are often poorly differentiated [14]. Zhang et al. also reported a significant relationship between *SALL4* and poor differentiation [3].

SALL4 expression was significantly associated with LVI (P = 0.033), though its relationship with nodal metastasis was not significant (P = 0.06). Conversely, Hao et al. reported a significant correlation between *SALL4* and lymph node metastasis, but they considered both cytoplasmic and nuclear staining as positive, unlike our study which considered only nuclear staining [15]. Differences in sample size and the small number of *SALL4*-positive cases in our study may also account for these variations. We found no significant relationship between *SALL4* expression and pT stage (P = 0.450) or PNI (P = 0.477). Similarly, Zhang et al. found no significant link with depth of invasion [3]. No literature was found regarding *SALL4* and PNI in CRC.

For *fascin*, 38.9% of cases showed moderate-to-high expression. This upregulation in CRC compared to normal tissue is consistent with a meta-analysis by Shi et al. [12].

We found a significant association between high *fascin* expression and advanced pT stage (P = 0.023), aligning with Tampakis et al., who reported significantly elevated *fascin* in stages III/IV CRC and lower expression in T1 tumors [6]. We observed no significant relationship between *fascin* expression and tumor grade (P = 0.317), consistent with Piskor et al. [16], though Mohammed et al. reported a significant association [9].

High *fascin* expression was significantly associated with lymph node metastasis (P = 0.031) and LVI (P = 0.01), but not with PNI (P = 0.204). These findings are supported by Tampakis et al. and Ozerhan et al. [6, 17].

Critically, we demonstrated a significant association between *SALL4* positivity and high *fascin* expression (P = 0.009), suggesting a potential interplay in CRC. To our knowledge, this relationship has not been previously reported. This result aligns with Barnawi et al., who found that *fascin* promotes the expression of stem cell transcription factors in breast cancer [2].

Conclusion and Limitations

The expressions of *SALL4* and *fascin* are significantly interrelated in CRC patients, suggesting a possible interplay between them. Moreover, their expression may represent potential markers for CRC progression. The relatively small sample size, the limited number of *SALL4*-positive cases, and the borderline significance of some results are limitations of this study. Further studies on larger cohorts are required to better assess this relationship. Molecular investigations are necessary to elucidate the underlying mechanisms in greater detail.

Author Contribution Statement

Study design: Nagwa M. Abdel-Rhaman. Data interpretation: Aya M. El-Iraqi & Azza Kamal Taha. Drafting of the manuscript: Aya M. El-Iraqi & Azza Kamal Taha. Final revision of the manuscript: Nagwa M. Abdel-Rhaman, Aya M. El-Iraqi & Azza Kamal Taha

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Ethical consideration

This retrospective study was conducted in accordance with the ethical standards of the Research Ethics Committee of the Faculty of Medicine for Girls, Al-Azhar University, and the 1964 Declaration of Helsinki and its subsequent amendments. The committee approved this study and waived the requirement for informed consent (IRB: 2023071978, dated May 6, 2025).

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